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Waterlogging Tolerance and Recovery in Canopy Development Stage of Cassava (*Manihot esculenta* Crantz)

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ABSTRACT

Cassava is susceptible to waterlogged soil. In order to find the right variety for breeding purposes, a research needs to find proper screening parameters. They must be easy, fast, and economical practice. Therefore, in this research, upper-ground morphological responses of cassava to water deficit conditions in tissue were evaluated as traits to screen cassava breeding lines for water-logging tolerance. Hanatee variety is a landrace grown in the well-watered field for cooking purpose and was bred with Kasetsart 50 which is a high yielding commercial variety. These two varieties together with five breeding lines have water-logging tolerance potential in the field. They were evaluated in the pot for waterlogging stress at two vegetative growth stages at 105 DAP and 165 DAP for 12 days in each stress period. Among these seven varieties/lines, there were no varieties/lines showing waterlogging tolerance under this condition over others, but all showing recovery response. The results indicated that cassava at the vegetative growth stage had a recovery mechanism for the upper-ground parts, but not for the storage root tissue after waterlogging stress for 12 days. There was a potential of using the ratio of leaf retention to screen cassava germplasm or breeding lines for waterlogging tolerance.

INTRODUCTION

Waterlogging is one of the major abiotic causes of constraints in crop production worldwide as climate change increases in frequency and severity and becomes more unpredictable. Up to 50% loss of cereal and legume production due to waterlogging happened around 10-16% of agricultural areas in Australia, the United States, Russia, and in many countries in Asia such as India, Pakistan, Bangladesh and China (Manik et al., 2019; Ploschuk, Miralles, Colmer, Ploschuk, & Striker, 2018). The major effects of waterlogging to plants are oxygen depletion together with other long-term consequences in the soil biological, chemical, and structure such as a reduction in aerobic microbial activities, soil pH and redox potential which causing nutrient deprivation and ion toxicity for plants and increasing soil compaction and bulk density (Phukan, Mishra, & Shukla, 2016).

For the effects of waterlogging on plant growth and development, there are two types of stresses, which are hypoxia and anoxia. Hypoxia effect, usually is caused by the excess of rainfall or seawater in the land, the plant is partially submerged in water mostly in the root zone, while; in anoxia effect, more often was caused by the flash flooding making a whole plant submerged in water. Plants have adapted to these effects variously and specifically in each species (Ahmed et al., 2013). In rice, there are two types of adaptation called Low Oxygen Escape Syndrome (LOES) in deep water varieties to elongate shoot over water level and Low Oxygen Quiescence Syndrome (LOQS) in Indian rice landrace which can hibernate under water in anaerobic metabolic condition for around two weeks and can regain growth after flooding (Hattori et al., 2009). In crop species, cereal and monocot crops tend to have more adaptive ability to waterlogging than legume and other dicot crops. Other than anaerobic respiration, plants have other

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mechanisms for waterlogging adaptation such as aerenchyma formation, adventitious root formation, and antioxidation. Ethylene is a phytohormone which initiates the formation of aerenchyma and adventitious root in the plants. It has functions to retain oxygen supply and induce leaf senescence and epinasty to reduce water transpiration. This condition indicates water deficit response in the upper grown part of plant due to the malfunction of root system under anaerobic condition (Colmer & Voesenek, 2009). In breeding program for waterlogging tolerance in rice, waterlogging parameters such as anaerobic germination, the rate of internode elongation and survival rate were used for screening lines for QTL evaluation instead of physiological traits (Oladosu et al., 2020).

However, in cassava, there has been no report of waterlogging tolerant variety or breeding program for waterlogging tolerance. Cassava is reported to be a highly adapted crop in drought-prone and nutrient insufficient areas (Jarvis, Ramirez-Villegas, Campo, & Navarro-Racines, 2012), but not in waterlogging areas because a severe reduction in root affects directly from rapid carbohydrate utilization through anaerobic respiration and root cell death. It is due to waterlogging and indirectly from root rot disease from fungi after waterlogging (Afolabi, Okechukwu, Kehinde, & Okechukwu, 2011; Sankar, Nath, Misra, & Lajapathy Jeeva, 2013). Therefore, cassava growers in areas tend to be subjected to waterlogging usually manage by building ridges or mounds for cassava plantations (Mohamoud, 1994). Moreover, if heavy rainfall or flooding comes when the cassava plant is over 6 months, farmers tend to harvest it abruptly to avoid the problem of root rot. Therefore, screening for cassava germplasm/breeding lines for the late developmental stage for waterlogging is not necessary for farmers. However, Thai farmers particularly grow cassava in the early rainy season, and, in some areas, waterlogging can occur in the late rainy season when cassava is in the stage of canopy establishment and root development (Ratanawaraha, Senanarong, & Suriyapan, 2001). According to Alves's (2002) explanation of cassava developmental stages, after planting cassava stake, it usually takes 30 days for primary leaves and root system establishment using carbohydrate source from mainly stake. After 30 days after planting (DAP), the first photosynthesis leaf and fibrous root system begin to develop. The storage root accumulation begins around 75 DAP. From 90 to 180 DAP; it is the stage of canopy establishment and root development to reach the maximum plant growth before switching to root accumulation stage after 6 months after planting. During canopy and root system establishment, if water deficit occurs, cassava tends to lose yield more than being subjected to water deficit in any other stages up to 32-60% (Alves, 2002). The fast phenotyping trait for water deficit adaptation in cassava was leaf characteristics. Zhao et al. (2015) reported that two varieties of cassava subjected to drought for 18 days could recover. However, if the water deficit period was extended to 35 days, only cassava variety showing wilting symptoms, it stays green and survive; while, other variety with more senescence leaves could not survive the 35-day water deficit period. Therefore, screening of leaf characteristics in response to water deficit due to waterlogging in the root zone of cassava breeding lines for waterlogging tolerance in the canopy developmental stage is important and suitable for breeding of high potential of waterlogging tolerance cassava for farmer utilization.

This study aimed to evaluate cassava morphological changes in response to waterlogging and recovery after stress in two canopy developmental stages at 105 DAP and 165 DAP and to indicate the leaf characteristic traits relating to root yield for further application in cassava breeding program for waterlogging tolerance.

MATERIALS AND METHODS

Plant Materials

Five inches-long Cassava stakes of Hanatee variety and Kasetsart 50 variety with 5 breeding lines of KU50 and HNT, namely, 55-161, 55-663, 55-695, 55-764, and 55-752 were planted in the germinating bag for 1 month. Afterward, cassava plants with fully germinating shoots and roots were transferred to 30-cm diameter pots for one plant per pot. Fertilizer formula 15-15-15 was applied 45 days after planting (DAP) at the rate of 312.5 kg/ha (31.25 g per plant).

Experimental Design and Conditions

The experiment was conducted at the Department of Agronomy, Faculty of Agriculture, Kasetsart University, Thailand from July 2018 until January 2019. The experimental design of this research was factorial in a randomized complete block design (RCBD) with 4 replications and one-pot per experimental unit. The factorial factors were soil moisture conditions and varieties/lines. For soil moisture conditions, there were three conditions as control groups with the normal practice of watering every four days for 10 mm (equal to 700 ml according to pot diameter area) coding as C, group subjected to waterlogging at 105 days after planting coding as W1, and group subjected to waterlogging at 165 DAP coding as W2. For each waterlogging period, four inches of water level above the soil surface were held for 12 days by putting cassava pots in the water container. All cassava plants were harvested at 185 DAP.

Data Collection

Plant height was measured in centimeters every seven days from the cassava age of 3 months until 6 months.

Leaf greenness was determined by chlorophyll meter (SPAD) (Minolta). Data collection was done from 4 pm to 6 pm in the 10th leaf from the top which was the first fully expanding leaf one day after the plant was subjected to waterlogging conditions.

The number of green leaves and the total number of leaves including yellow leaves were counted every 3 days after plants were subjected to waterlogging. The ratios of green leaves per total leaves were calculated as one of the parameters for determining waterlogging tolerance.

The relative water content of the 10th leaf (the same leaf that was measured for leaf greenness), at one day after the plant was subjected to waterlogging condition, was analyzed by weighing fresh leaf weight (FW) immediately and then leaf sample was transferred to a container filled with distilled water overnight. On the next day, the leaf sample was weighed again for the full water-absorbing weight (TW) and then the leaf sample was dried in a 70°C hot-air oven for six hours. Afterwards, dried leaf weight was measured (DW). Relative water content (RWC) was calculated in percentage as follows (Soltys-Kalina, Plich, Strzelczyk-Żyta, Śliwka, & Marczewski, 2016):

$$\mathrm{RWC}\ (\%) = \frac{\mathrm{FW} - \mathrm{DW}}{\mathrm{TW} - \mathrm{DW}} \times 100 \quad \text{.................................. 1)}$$

Where:
FW = Fresh leaf weight;
DW = Dried leaf weight;
TW = fully water-absorbing weight

Fresh root and shoot weight were collected at 185 DAP by weighing separately. Harvest index (HI) was calculated as follows:

$$\mathrm{HI} = \frac{\text{Fresh root weight}}{\text{Total cassava plant weight}} \quad \text{.............................. 2)}$$

Statistical Analysis

The data of each trait were analyzed for analysis of variance (ANOVA) using factorial in RCBD and were then analyzed for the phenotypic correlation coefficients by the Pearson correlation coefficients (r) among the agronomic traits by The Statistic Tool for Agricultural Research STAR 2.0.1 software (IRRI, 2013). Under STAR software, Duncan's multiple range test was performed for mean comparison among factors. Broad-sense heritability (H2) was calculated from mean square values from ANOVA as shown in this formula from Hallauer & Miranda Filho (1988):

$H^2 = \sigma^2 g / \sigma^2 p$ ………….............……............…… 3)

Where:

$\sigma^2 g$ is genotypic variance and $\sigma^2 p$ is phenotypic variance

$\sigma^2 g = (MS_G – MS_{GE}) / (r * e)$……………….........….. 4)

Where:

MS_G is mean square of variety/lines and MS_{GE} is mean square of interaction between lines and treatment, r is the number of replications and e is the number of environments (treatments).

$\sigma^2 p = \sigma^2 g + (\sigma^2_{GE})/e + (\sigma^2 e)/(r*e)$ ……………...… 5)

Where:

σ^2_{GE} is variance of interaction and $\sigma^2 e$ is mean square error (MSe).

$\sigma^2_{GE} = (MS_{GE} – MSe) / r$ ……………….............….... 6)

RESULTS AND DISCUSSION

From 2 months to 6 months after planting, cassava is in the major shoot developmental stage. (Alves, 2002). Cassava plant height is one of the parameters indicating vegetative growth which will reach the maximum height after the canopy establishment stage when the stem starts to be lignified (Alves, 2002). Aina, Dixon, & Akinrinde (2007) reported that plant height trait was sensitive to water deficit conditions under drought treatments in the greenhouse and in the field with a reduction of up to 47%. In Table 1, cassava plant heights were measured from cassava developmental stages from three to six months old. At 105 days after planting (DAP) and 165 DAP, cassava plants in W1 and W2, respectively, were subjected to waterlogging for 12 days. Waterlogging did not have an effect on cassava plant height. There was no significant difference among heights of cassava plants in control and waterlogging-subjected groups from age ranging from 95 DAP to 172 DAP which covering both 12-day waterlogging periods at 105 DAP and 165 DAP. Comparing among varieties, cassava line number 695, 161, and 752 were in the highest group from 95 DAP to 172 DAP. However, HNT variety was a fast-growing variety during these periods which made it had the same height as the highest groups at 172 DAP. Among these varieties, line 764 had the lowest height. There was no significant interaction between the treatment of waterlogging factor and the variety factor which made these ranks of the height of each variety were the same in all waterlogging treatments. Broad-sense heritability of plant height at different stages was in the range of 0.901-0.947 that indicates that the performance of cassava height was not affected by water deficit in tissue due to waterlogging. Therefore, plant height could not be used to evaluate waterlogging tolerance in cassava at the vegetative developmental stage. Moreover, this result indicates that waterlogging for 12 days did not destroy cassava shoot meristem. For this reason, the regular increase rate of cassava height could continue during and after waterlogging treatments. Turyagyenda et al. (2013) also reported that the height of cassava under drought stress did not show significant difference from those in well-watered conditions.The suggestion for a more sensitive trait for water deficit to plant cell expansion and proliferation is the expansion of unfolding leaves subjected to water deficit condition. Alves & Setter (2004) suggested that eight days of soil water stress could trigger the reduction of cell proliferation; cell expansion and delay of these processes in unfolding leaves after a re-watering period. According to Alves & Setter (2004), non-fully expanded leaves were classified into three groups counting from base to apex which were group 1, comprising of leaf 1 to 5 which unfolded already, but still not become fully expanded ones yet due to the continuing of the cell expansion process, group2, comprising of leaf 6 to 11 which were still folded leaves with undergoing a process of cell proliferation and cell expansion, and group 3, comprising of leaf 12 to 15 which were still in the meristem stage. Among these three groups of incompletely expanded leaves, group 2 showed the most sensitive response to water deficit due to 50% of size reduction after re-watering. Therefore, during the recovery period, the size of leaves in group 2 compared to control, instead of plant height should be tested for the potential to be used to evaluate plant response to water deficit in the screening process of the breeding program.

Stay green leaf is one of the parameters for evaluating of plant adaptation to water deficit. Zhao et al. (2015) showed that two cassava varieties having different response of leaf to water deficit had the different tolerance level considering in the recovery phase after re-watering. Variety with wilting, but stay green leaves, could recover after 35 days of soil moisture stress; while, variety with yellow leaf senescence could not survive after 35 days of water deficit. Therefore, this research assumed that if a plant contained more green leaves, it might show more water deficit adaptive level compared to ones with fewer green leaves. Table 2 shows the number of green leaves in cassava in all treatment groups in two period of waterlogging at 105 DAP for W1 group and at 165 DAP for W2 group. During waterlogging treatment at 105 DAP, the significant difference between the number of green leaves in cassava in waterlogging treatment and those in control treatment just occurred at 12 days after plants were subjected to waterlogging condition. At 12 days of waterlogging, the cassava plant in W1 group had a total number of the green leaf around 7 leaves; while, the control plant had 15 leaves. Remarkably, during waterlogging treatment of the older plant at 165 DAP, the significant difference between the number of green leaves in cassava in waterlogging treatment and those in control treatment occurred sooner at 6 days after plants were subjected to waterlogging condition. At 6 days of waterlogging, the cassava plant in W2 group had a total number of green leaf around 6 leaves; while, control plant had 22 leaves. This result indicates that cassava plants at an early stage (105 DAP) of canopy development tended to have more water deficit adaptability than those in the late stage (165 DAP) as mentioned in Alves (2002) that canopy establishment would reach a maximum during 120 to 150 DAP. For this reason, after 150 DAP, there would be less leaf emergence. According to the leaf longevity period, cassava leaf tends to stay after fully expanding for 36 to 100 days (Alves, 2002). Thus, cassava leaves at 165 DAP in this experiment were in the mature stage which tended to be subjected to leaf senescence more than those in 105 DAP. Moreover, plants in the group subjected to waterlogging at 105 DAP had the recovery mechanism showing in the number of green leaves in this group which was not significantly different from those in the control group at 165 DAP.

Table 1. Cassava plant height under treatments of waterlogging and control

Factor	95 DAP	105 DAP	112 DAP	126 DAP	148 DAP	165 DAP	172 DAP
Treatment (Tr)							
C	35.93	37.43	40.25	42.18	47.82	51.00	53.00
W1	35.80	37.52	39.15	40.06	44.25	47.43	48.77
W2	38.28	39.68	42.64	44.15	49.51	52.41	53.78
P-Value	ns	ns	ns	ns	ns	ns	ns
Cultivar (Cul)							
663	35.75 bcd	37.50 bc	38.83 bcd	40.00 bcd	44.37 bcd	47.16 b	48.40 b
695	44.58 ab	45.58 ab	46.50 abc	47.17 abc	48.79 abc	49.85 ab	52.10 ab
161	49.17 a	50.58 a	52.58 a	53.83 a	58.08 a	60.08 ab	61.50 a
752	41.00 abc	43.17 abc	47.92 ab	50.50 ab	56.08 ab	60.42 a	62.42 a
764	24.58 d	25.50 d	27.50 d	28.92 d	31.81 d	33.50 c	34.72 c
KU50	30.67 cd	32.58 cd	35.25 cd	36.17 cd	43.08 cd	47.38 ab	48.45 b
HNT	31.83 cd	33.67 cd	36.58 bcd	38.33 bcd	44.77 bc	50.00 ab	52.02 ab
P-Value	**	**	**	**	**	**	**
Tr*Cul							
P-Value	ns	ns	ns	ns	ns	ns	ns
% CV	24.51	23.87	23.52	23.4	21.96	21.12	20.17
H^2	0.943	0.937	0.947	0.937	0.901	0.906	0.908

Remarks: ** = Significant at the 0.01 probability level, ns = Non-significant; DAP = days after planting; C = control group, W1 = waterlogging group treated at 105 DAP, W2 = waterlogging group treated at 165 DAP; H^2 = broad-sense heritability of traits

Interestingly, line 764 which had the lowest height (Table 1) had significantly more green leaves than HNT and KU50 (Table 2) which had higher shoot parts. Even under waterlogging stressed conditions, line 764 still had more green leaves than HNT and KU50 as shown in non-significant interaction between variety factor and waterlogging treatment factor. However, regarding Alves (2002), the total leaves of the cassava plant should be in the range of 44 to 146 leaves, but the number of green leaves in this pot experiment at 177 days which should be the end of the canopy establishment stage had an average leaf number around 21 leaves or in line 764 which had the highest leaf number still had only 30 leaves. Therefore, the condition in the pot might have some limitations for plant growth. Moreover, considering the significant interaction at the period of waterlogging at 105 DAP (W1) for 3 days and 165 DAP (W2) for 12 days (Table 2), broad-sense heritability of the number of green leaf counted per plant at 3 days of waterlogging in W1 stage (H^2 = 0.614) and at 12 days of waterlogging in W2 stage (H^2 = 0.679) were lower than others (H^2 range from 0.736 to 0.879) indicating the different performance of genotypes in different treatments which also be shown in the significant interaction between variety and treatment factors in Table 2.

Considering only the number of green leaves *per se* might mislead the evaluation due to various plant heights among varieties have leaf abundance. Therefore, the ratio of green leaves per total attaching leaves including yellow leaves was used to evaluate plant senescence in this experiment as shown in Table 3. During waterlogging treatment at 105 DAP, the significant difference between the ratio of green leaves per total leaves in cassava in waterlogging treatment and those in control treatment occurred at 6 days after plants were subjected to the waterlogging condition. This ratio showed significant difference sooner than the number of green leaves *per se* in which significant difference occurred at 12 days in waterlogging. Moreover, during waterlogging treatment of the older plant at 165 DAP, the significant difference between the ratio of green leaves per total leaves in cassava in waterlogging treatment and those in the recovering group from waterlogging at 105 DAP started to occur at 3 days after plants were subjected to waterlogging condition.

Table 2. Number of green leaves per plant under treatments of waterlogging and control

Factor	3 DS-W1	6 DS-W1	9 DS-W1	12 DS-W1	3 DS-W2	6 DS-W2	9 DSW2	12 DS-W2
Treatment (Tr)								
C	12.21	11.89	12.57	12.25 a	17.46	19.36 a	19.86 a	21.07 a
W1	15.09	11.50	11.24	7.16 b	17.99	19.89 a	20.78 a	22.20 a
W2	14.06	14.14	14.27	14.36 a	19.30	13.61 b	9.32 b	6.02 b
P-Value	ns	ns	ns	**	ns	**	**	**
Cultivar (Cul)								
663	12.47 abc	11.08 bcd	10.67 bc	9.00 bc	16.34 bc	15.28 b	14.65 b	15.65 b
695	16.53 ab	15.83 ab	16.25 ab	13.58 ab	17.31 bc	16.45 b	16.02 b	15.44 b
161	14.92 ab	14.25 abc	14.08 ab	12.92 ab	19.33 b	19.33 b	17.17 b	16.58 b
752	15.39 ab	13.25 abc	13.42 ab	12.50 ab	18.15 bc	16.50 b	15.25 b	15.47 b
764	20.16 a	18.17 a	18.17 a	16.58 a	32.97 a	30.88 a	30.93 a	30.68 a
KU50	10.11 bc	9.00 cd	10.04 bc	9.80 bc	14.84 bc	14.34 b	13.31 b	12.85 b
HNT	6.94 c	6.17 d	5.67 c	4.41 c	8.81 c	10.55 b	9.24 b	8.35 b
P-Value	**	**	**	**	**	**	**	**
Tr*Cul								
P-Value	*	ns	ns	ns	ns	ns	ns	*
% CV	43.25	42.09	45.17	46.43	41.95	46.6	48.08	47.05
H^2	0.614	0.736	0.765	0.869	0.879	0.855	0.832	0.679

Remarks: * = Significant at the 0.05 probability level, ** = Significant at the 0.01 probability level, ns = Non-significant; C = control group, W1 = waterlogging group treated at 105 DAP, W2 = waterlogging group treated at 165 DAP, DS = number of days plants subjected to waterlogging condition (for indicated treatment group after dash sign); H^2 = broad-sense heritability of traits

Meanwhile, waterlogging cassava at 165 days showed a significant difference in the ratio of green leaves per total leaves from those in the control group at 6 days after subjected to waterlogging stress. Among varieties, HNT showed a low ratio of green leaves per total leaves throughout the period especially in the waterlogging at 105 DAP for 12 days that HNT showed the significantly lowest ratio of green leaves per total leaves. This result also coincided with the lowest number of green leaves of HNT in Table 2. However, in waterlogging at 165 DAP, HNT did not show a significantly lower ratio of green leaves per total leaves in the late period of waterlogging. Moreover, there was significant interaction at 12 days under waterlogging conditions. HNT in W2 had no significantly lower ratio of green leaves per total leaves than one another. On the other hand, line 764 which had a high number of green leaves had the lowest ratio of green leaves per total leaves. Considering the number of green leaves of line 764 in W2 group, line 764 had no significantly different number of green leaves from other varieties/lines. This result indicates that cassava might have the minimum amount of green leaves which were necessary for maintaining a whole plant system. Therefore, even though line 764 had more number of leaves on regular basis, it had to shred some off during water deficit condition to reach the minimum number of adequate green leaves which was around 3 to 8 leaves for all varieties/lines in this experiment as shown in Table 2. However, for plants in the control treatment and W1 treatment which were not subjected to waterlogging at W2 stage as plants in W2 group. The leaf senescence rarely occurred in both control and W1 group causing low mean square value of variety which led to the non-significant difference among varieties and lines. It is also seen that the low or negative value of broad-sense heritability at day 6 to 12 under waterlogging in W2 stage because of the value of the mean square of variety was lower than that of interaction. These traits could be used to distinguish treatment which was subjected to waterlogging from other treatment sooner than using the number of green leaf *per se*. The green leaf retention was reported as one of the parameters for drought tolerance screening (Okogbenin et al., 2013).

Table 3. Ratio of green leaves per total leaves under treatments of waterlogging and control

Factor	3 DS-W1	6 DS-W1	9 DS-W1	12 DS-W1	3 DS-W2	6 DS-W2	9 DS-W2	12 DS-W2
Treatment (Tr)								
C	0.87	0.80 a	0.82 a	0.77 a	0.96 ab	0.98 a	0.99 a	0.99 a
W1	0.92	0.74 b	0.65 b	0.60 b	0.98 a	0.99 a	0.99 a	0.99 a
W2	0.92	0.86 a	0.85 a	0.83 a	0.92 b	0.72 b	0.58 b	0.75 b
P-Value	ns	**	**	**	*	**	**	**
Cultivar (Cul)								
663	0.97 a	0.85 ab	0.78 ab	0.72 a	0.99 ab	0.96	0.92	0.93
695	0.92 ab	0.86 ab	0.87 a	0.73 a	0.99 a	0.92	0.87	0.95
161	0.86 b	0.80 abc	0.81 ab	0.80 a	0.95 abc	0.92	0.83	0.87
752	0.88 ab	0.76 abc	0.75 ab	0.71 a	0.91 bc	0.83	0.89	0.97
764	0.94 ab	0.89 a	0.86 a	0.84 a	0.97 abc	0.86	0.82	0.78
KU50	0.84 b	0.73 bc	0.71 ab	0.81 a	0.97 abc	0.89	0.85	0.95
HNT	0.88 ab	0.71 c	0.65 b	0.52 b	0.89 c	0.88	0.85	0.95
P-Value	**	**	**	**	**	ns	ns	ns
Tr*Cul								
P-Value	*	ns	ns	ns	ns	ns	ns	*
%CV	8.7	14.23	17.19	18.15	6.35	15.64	15.88	23.13
H^2	0.389	0.594	0.763	0.877	0.685	0.084	-0.341	-0.231

Remarks: * = Significant at the 0.05 probability level, ** = Significant at the 0.01 probability level, ns = Non-significant; C = control group, W1 = waterlogging group treated at 105 DAP, W2 = waterlogging group treated at 165 DAP; DS = number of days plants subjected to waterlogging condition (for indicated treatment group after dash sign); H^2 = broad-sense heritability of traits

However, for varieties/breeding lines in this experiment, there were no varieties/lines which had higher green leaf number or leaf retention than one another. Zhang et al. (2010) had transgenic cassava with isopentenyl transferase (*IPT*) from *Agrobacterium tumefaciens* induced by senescence on SAG12 promoter that showed stay-green phenotype under drought condition by maintaining cytokinin homeostasis in plant. The stay-green phenotype which was suitable for drought adaptation was also confirmed in Turyagyenda et al. (2013) and Zhao et al. (2015) that genotype with stay-green phenotype could tolerate and recover from drought much better than those with leaf senescence.

Leaf greenness and relative water content at the fully unfold leaf were measured on the second day after the plants subjected to waterlogging at 105 DAP and 165 DAP as shown in Table 4. For fully unfold leaf, according to Alves & Setter (2004), the 15th leaf from top should be collected and regarded as the first fully expanded leaf. However, in the pot experiment, some samples have total number of leaves fewer than 15 leaves. Therefore, the 10th leaf of each plant was collected and regarded as fully unfold leaf, but not the first fully expanded leaf. In fully unfold leaves, cell expansion was continued, and photosynthesis structure was establishing. Therefore, if water deficit occur, nitrogen could not be transport to this leaf for chlorophyll synthesis which can be measure as leaf greenness (Barutcular, Toptas, Turkten, Yildirim, & Koc, 2015). Unay & Simsek (2020) had studied heritability of chlorophyll concentration (SPAD) and chlorophyll content index (CCI) in wheat both under waterlogging and control condition. They found that waterlogging had the effect on both chlorophyll parameters which had lower heritability than those in control group. However, there was no significant difference in leaf greenness among waterlogging stress conditions and control in this experiment. Only the relative water content at 165 DAP waterlogging showed a significant difference from those in control. At 105 DAP, broad-sense heritability of relative water content was negative due to the high value of mean square error which also caused non-significant difference among varieties and treatment for this trait. Turyagyenda et al. (2013) suggested that the decreasing of relative water content in leaf under drought stress indicating the wilt symptom in the plant. Fresh root weight and harvest index of cassava measured at 185 DAP all showed significant difference among groups subjected to waterlogging at both 105 and 165 DAP comparing to the control group. Plants in W1 were subjected to waterlogging at 105 DAP, but fresh root weight was the same as those in plants in the W2 group which were subjected to waterlogging at 165 DAP. Therefore, this result can be interpreted that waterlogging for 12 days could destroy cassava storage root immediately and destroyed storage roots could not be repaired. Nevertheless, the cassava root system for water and nutrient absorption was not destroyed in the 12-day waterlogging period so that plant could recover after waterlogging. The fresh shoot weights of cassava in three groups, however, were not significantly different. These results indicate that waterlogging affected cassava root development but did not affect shoot development. For this reason, it could be concluded that recovery in shoot growth could occur after 12 days of waterlogging as also shown in regular plant height after waterlogging, but not in root growth especially for the storage root. The reason for the reduction of storage root weight might due to the utilization of storage carbohydrates as a source for anaerobic respiration under the waterlogging condition of the root. Moreover, the root rot symptom was seen when harvesting indicating the death of root tissue causing soil microbes to come to use root carbohydrate as the source of energy for anaerobic respiration as well.

Table 4. Leaf greenness (SPAD unit), relative water content (%) in leaf, root and stem weight (g per plant) and harvest index (HI) of cassava under treatments of waterlogging and control

Factor	SPAD 1	SPAD 2	RWC 1 (%)	RWC 2 (%)	Root weight (g per plant)	Stem weight (g per plant)	HI
Treatment (Tr)							
C	26.69	28.91	0.92	0.87 a	36.46 a	73.21	0.29 a
W1	25.5	26.57	0.85		14.04 b	71.57	0.13 b
W2		28.14		0.81 b	21.21 b	70.02	0.19 b
P-Value	ns	ns	ns	**	**	ns	**
Cultivar (C)							
663	25.70	33.07 a	0.92	0.87	27.50 ab	50.08 c	0.29 a
695	29.90	31.63 ab	0.88	0.83	40.92 a	96.33 a	0.21 ab
161	27.51	28.02 bc	0.92	0.84	37.92 a	87.58 ab	0.28 ab
752	25.84	29.53 abc	0.85	0.88	15.42 ab	69.00 abc	0.15 ab
764	28.30	24.40 cd	0.91	0.79	14.58 ab	56.08 bc	0.17 ab
KU50	26.49	26.23 cd	0.89	0.81	21.08 ab	57.08 bc	0.19 ab
HNT	18.96	22.21 d	0.84	0.84	9.92 b	66.92 abc	0.11 b
P-Value	ns	**	ns	ns	**	**	*
Tr*C							
P-Value	*	ns	ns	ns	ns	ns	ns
%CV	19.13	16.92	14.91	8.81	90.7	37.84	68.89
H^2	0.740	0.947	-0.732	0.508	0.683	0.625	0.521

Remarks: * = Significant at the 0.05 probability level, ** = Significant at the 0.01 probability level, ns = Non-significant; SPAD 1 and SPAD 2 measured one day after waterlogging at 105 DAP and 165 DAP, respectively; RWC 1 and RWC 2 measured one day after waterlogging at 105 DAP and 165 DAP, respectively; H^2 = broad-sense heritability of traits

Table 5. Correlation between morphological traits and cassava root and stem yield and harvest index at harvesting period

r p-value	Root (C)	Stem (C)	HI (C)	Root (W1)	Stem (W1)	HI (W1)	Root (W2)	Stem (W2)	HI (W2)
Height at 95 DAP	0.645**	0.773**	0.227ns	0.431*	0.681**	0.238ns	0.562**	0.713**	0.362ns
Height at 105 DAP	0.614**	0.767**	0.208ns	0.433*	0.699**	0.230ns	0.504**	0.716**	0.297ns
Height at 112 DAP	0.548**	0.708**	0.195ns	0.429*	0.713**	0.230ns	0.409*	0.679**	0.212ns
Height at 126 DAP	0.489**	0.682**	0.136ns	0.426*	0.712**	0.231ns	0.377*	0.646**	0.195ns
Height at 148 DAP	0.481*	0.679**	0.134ns	0.275ns	0.667**	0.002ns	0.310ns	0.670**	0.117ns
Height at 165 DAP	0.403*	0.640**	0.068ns	0.309ns	0.686**	0.038ns	0.197ns	0.586**	0.023ns
Height at 172 DAP	0.381*	0.626**	0.049ns	0.299ns	0.682**	0.032ns	0.171ns	0.583**	-0.003ns
SPAD 1	0.311ns	0.267ns	0.069ns	0.417*	0.128ns	0.363ns	-	-	-
SPAD 2	0.524**	0.360ns	0.304ns	-	-	-	0.496**	0.282ns	0.496**
RWC 1	0.254ns	0.161ns	0.236ns	0.035ns	0.018ns	-0.011ns	-	-	-
RWC 2	-0.530*	-0.396*	-0.385*	-	-	-	-0.378ns	-0.050ns	-0.283ns
Green (3)1	0.712**	0.713**	0.301ns	0.383*	0.311ns	0.366ns	0.207ns	0.258ns	0.241ns
Green (6)1	0.709**	0.701**	0.322ns	0.338ns	0.216ns	0.333ns	0.366ns	0.331ns	0.318ns
Green (9)1	0.662**	0.643**	0.283ns	0.389*	0.284ns	0.365ns	0.361ns	0.312ns	0.340ns
Green (12)1	0.619**	0.555**	0.309ns	0.397*	0.529**	0.270ns	0.312ns	0.271ns	0.275ns
Ratio (3)1	0.050ns	0.135ns	-0.033ns	0.255ns	0.062ns	0.427*	0.042ns	-0.039ns	0.211ns
Ratio (6)1	0.220ns	0.168ns	0.140ns	0.240ns	-0.019ns	0.305ns	0.355ns	0.042ns	0.457*
Ratio (9)1	0.072ns	0.119ns	-0.020ns	0.397*	0.473*	0.431*	0.480*	0.183ns	0.566**
Ratio (12)1	0.107ns	0.021ns	0.170ns	0.009ns	0.111ns	-0.151ns	0.459*	0.092ns	0.492**
Green (3)2	0.237ns	0.104ns	0.234ns	0.585**	0.585**	0.445*	0.208ns	0.197ns	0.220ns
Green (6)2	0.156ns	0.116ns	0.136ns	0.561**	0.578**	0.411ns	0.277ns	0.247ns	0.287ns
Green (9)2	0.195ns	0.054ns	0.212ns	0.541*	0.552**	0.408ns	0.121ns	0.149ns	0.173ns
Green (12)2	0.135ns	-0.007ns	0.167ns	0.506*	0.525*	0.385ns	-0.057ns	0.070ns	0.057ns
Ratio (3)2	0.132ns	0.225ns	0.033ns	0.337ns	0.178ns	0.334ns	0.424*	0.059ns	0.485**
Ratio (6)2	-0.011ns	0.079ns	-0.088ns	-0.092ns	-0.168ns	0.016ns	0.319ns	0.111ns	0.355ns
Ratio (9)2	0.167ns	0.198ns	0.172ns	0.217ns	0.020ns	0.307ns	-0.114ns	-0.075ns	0.012ns
Ratio (12)2	0.286ns	0.129ns	0.448*	0.158ns	-0.030ns	0.141ns	-0.122ns	0.042ns	-0.069ns

Remarks: * = Significant at the 0.05 probability level, ** = Significant at the 0.01 probability level, ns = Non-significant; Green means the number of green leaves per plant; Ratio means the ratio of green leaves per total leaves; The number 1 or 2 indicates measurement period during waterlogging at 105 or 165 DAP, respectively; The number in parenthesis indicates the number of days during waterlogging treatment.

For screening traits in the breeding programs, the major purpose of screening is to select a plant with stress adaptation and maintaining plant yield as well. Therefore, the traits studied in this experiment must be analyzed for the relationship between root yield, stem weight, and harvest index. From the correlation analysis (Table 5), the height of cassava from 95 DAP to 172 DAP had a positive correlation with stem weight harvested at 185 DAP in control group (C), group subjected to waterlogging at 105 DAP (W1), and group subjected to waterlogging at 165 DAP (W2) with correlation coefficient ranging from 0.583 to 0.773. Remarkably, the height of cassava also had a positive correlation with root weight. However, in the control group, there was a positive correlation between root weight and height of cassava from 95 DAP to 172 DAP; while, in two waterlogging stress groups (W1 and W2), there was a positive correlation between root weight and height of cassava as well, but only in the period of 95 DAP to 126 DAP. This result could be the damage of the root in the W1 and W2 group without the damage in the upper ground weight which made height at the late stage did not correlate with root weight. For harvest index, there was no correlation between plant height and harvest index.

Leaf greenness and relative water content of the fully expanding leaf, which were measured at 106 DAP which was the second day after plants were subjected to waterlogging at 105 DAP for waterlogging stress group, mostly had no correlation with root weight, stem weight, and harvest index except for leaf greenness of waterlogging stress group which had a positive correlation with root weight (with r = 0.417). For leaf greenness of the fully unfold leaf, which was measured at 166 DAP which was the second day after plants were subjected to waterlogging at 165 DAP for waterlogging stress group, there were positive correlations between leaf greenness and root weight in both control and the stressed group with waterlogging at 165 DAP with correlation coefficient 0.524 and 0.496, respectively. Interestingly, there was a positive correlation between leaf greenness and harvest index of stressed group with waterlogging at 165 DAP (with r = 0.496). For the relative water content of control group measured at 166 DAP, there were a negative correlation with root weight, stem weight, and harvest index.

The number of green leaves of control group measured at 108 DAP, 111 DAP, 114 DAP, and 117 DAP had positive correlation with root weight and stem weight; while, there were positive correlations between the number of green leaves of waterlogging stressed group and root weight and stem weight as well, but did not have continuing correlation from 108 DAP to 117 DAP as in control group. For the ratio of green leaf per total leaf, there was no correlation between this parameter and root weight, stem weight, and harvest index in control group. However, in waterlogging stressed group, there were positive correlation between this parameter at 114 DAP and root weight, stem weight, and harvest index. Moreover, during the period of 111 DAP to 117 DAP, the ratio of green leaf per total leaf of the group which was subjected to waterlogging at 165 DAP also had a positive correlation with root weight and harvest index.

For the number of green leaves and the ratio of green leaf per yellow leaf of the control group measured at 168 DAP, 171 DAP, 174 DAP, and 177 DAP, there was no correlation with root weight, stem weight, and harvest index except for the ratio at 177 DAP with harvest index (r = 0.448). For the number of green leaves and the ratio of green leaf per yellow leaf of waterlogging stressed group measured at 168 DAP, 171 DAP, 174 DAP, and 177 DAP, there was no correlation with root weight, stem weight, and harvest index except for the ratio at 168 DAP with root weight and harvest index (r = 0.424, 0.485 respectively). Interestingly, there was a positive correlation between the number of green leaves measured at 168 DAP, 171 DAP, 174 DAP, and 177 DAP of the group of cassava which was subjected to waterlogging at 105 DAP and root weight and stem weight of cassava harvested at 185 DAP indicating the recovery phase of this group.

CONCLUSION AND SUGGESTION

Cassava subjected to waterlogging during the early canopy development stage could recover during the late of this stage if it was subjected to waterlogging for 12 days. In this study, cassava plants at the early stage of canopy development tended to have more water deficit adaptability than those in the late stage. For breeding program, the ratio of green leaf number to total leaf number is suggested to validate as a parameter for screening cassava germplasm and breeding population for waterlogging tolerance.

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